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Temperature changes and the ATP concentration of the soil microbial biomass

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Abstract

Two soils from temperate sites (UK; arable and grassland) were incubated aerobically at 0, 5, 15 or 25°C for up to 23 days. During this period both soils were analysed for soil microbial biomass carbon (biomass C) and adenosine 5′ triphosphate contents (ATP). Biomass C did not change significantly in either soil at any temperature throughout, except during days 0 to 1 in the grassland soil. Soil ATP contents increased slowly throughout the 23 days of incubation, from 2.2 to a maximum of 3.1 nmol ATP g^{-1} soil in the arable soil (a 40% increase) and from 6.2 to a maximum of 11.2 nmol ATP g^{-1} soil in the grassland soil (an increase of 81%), both at 25°C. Since biomass C did not change either with increasing temperature or increasing time of incubation, it was concluded that an increase in ATP was either due to an increase in adenylate energy charge or de novo synthesis of ATP, or both. During the incubation, biomass ATP concentrations ranged from about 5 to 12 μmol ATP g^{-1} biomass C but trends between biomass ATP and incubation temperatures were not very obvious until about day 13. On day 23, biomass ATP concentrations were positively and linearly related to temperature: (μmol ATP g^{-1} biomass C = $6.98 \pm 0.35 + 0.134 \pm 0.023\ T^0$ ($r^2 = 0.77$) with no significant difference in the slope between the grassland and arable soils. At 25°C the biomass ATP concentration was 10.3 μmol g^{-1} biomass C, remarkably close to many other published values. It was concluded that, although the biomass increased its ATP concentration in response to increasing temperature, the increase was comparatively small. Also, at all temperatures tested, the biomass maintained its ATP concentration within the range commonly reported for micro-organisms growing expontentially in vitro. This is despite the fact that the biomass normally exhibits other features more typical of a "resting" or dormant population — a paradox which still is not resolved.

Keywords: Soil microbial biomass; Soil ATP and temperature; Biomass ATP concentration

1. Introduction

There is substantial evidence which suggests that the soil microbial biomass maintains an adenosine 5′ triphosphate (ATP) concentration typical of micro-organisms undergoing exponential growth in vitro. This high concentration (around 10 to 12 μmol ATP g^{-1} biomass C) is unchanged irrespective of whether the soil is unamended, where microbial metabolism is very slow (e.g., Jenkinson and Ladd, 1981), or if readily decomposable substrates, e.g., glucose or plant residues have been recently given, which can trigger intense microbial activity. (e.g., Jenkinson et al., 1979; Jenkinson, 1988; Joergensen et al., 1990; Ocio and Brookes, 1990; Chander and Brookes, 1991).

In some circumstances, this close and consistent relationship does not appear to hold. For example, De Nobili et al. (1996) reported biomass ATP concentrations as high as 35 μmol ATP g^{-1} biomass C 3 days after glucose addition to a UK grassland soil. How-

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ever, this was finally attributed to exocellular ATPase activity in the glucose-amended soil. This caused aberrantly low recoveries of ATP in the "spike", leading to overestimation of biomass ATP once the correction for soil ATP fixation was made.

Cheng-Sheng et al. (1997) also considered that biomass ATP concentrations increased systematically, by up to nearly 3-fold (from about 3.9 to 9.0 μmol ATP g^{-1} biomass C), during the first few days following glucose addition to a sandy-loam soil. However, their extraction method was much less vigorous and efficient than ultrasonics as used by others (e.g., Jenkinson and Oades, 1979). Since ATP is such a labile cellular constituent (Karl, 1980) it is essential that the degradative enzymes in the microbial cells are inactivated immediately or ATP will be dephosphorylated and lost. The higher biomass ATP concentrations during the first few days after glucose addition could therefore reflect, at least partially, more efficient extraction of ATP from younger than older cells due to the rather inefficient extraction procedure used.

Jenkinson and Ladd (1981) laid down stringent criteria which must be met if a method is to satisfactorily extract ATP from soil. These are that it must (1) release all the ATP from the biomass; (2) instantly inactivate all the ATP-hydrolysing enzymes in both the biomass and soil; and (3) hold the released ATP in solution against sorption on the soil colloids. In our experience, most results which differ considerably from the mean biomass ATP concentrations cited by Jenkinson (1988) for moist aerobic mineral soils, whether substrate-amended or unamended, usually result from a failure to meet one or more of the above criteria.

Some published data argues against a reasonably consistent concentration of ATP in the microbial biomass (e.g., Ross et al., 1981, 1990; Sparling et al., 1981; Sparling and Eiland, 1983). However, Jenkinson (1988) collated all relevant results in the world literature and found that ATP and biomass C concentrations were very highly correlated over about 45 soils, giving a mean biomass ATP concentration of 11.7 ± 0.29 μmol ATP g^{-1} biomass C. This was in moist aerobic mineral soils, which received a 'conditioning incubation' of 5–10 days at 25°C before analysis, prior to extraction with strongly acidic reagents under the criteria suggested by Jenkinson and Ladd (1981).

Under these conditions, our picture of the soil microbial biomass is as a large, mainly dormant community with, paradoxically, a high ATP concentration (Jenkinson and Oades, 1979) and adenylate energy charge (Brookes et al., 1983) equivalent to those in micro-organisms growing exponentially in pure culture in vitro. However, this conclusion is based almost exclusively on measurements made on soils given a 'conditioning incubation' under the above, standard, conditions, at 25°C. In contrast, the biomass in the natural environment normally survives under conditions of moisture and temperature which may fluctuate considerably and rapidly. For example, the subsurface temperature of UK soils may easily range from below 0° to more than 20°C (Payne and Gregory, 1988).

Our aims were to determine whether changes in temperature affected the amounts of ATP and biomass in soil and the ATP concentration of this biomass. This is particularly important because soils are typically given a "conditioning incubation" at 25°C for 7–10 days before biomass measurements. If this incubation significantly changes the concentration of biomass or ATP in soils from that in the population in the field, this would seriously affect the interpretation of measurements of the soil microbial biomass.

2. Materials and methods

2.1. Soils

Soils were sampled from two field plots of the Highfield Long Term Ley-Arable Experiment at Rothamsted (UK). Soil 1 has been under permanent arable and Soil 2 under permanent grassland, both since 1896 (Johnston, 1973). Both soils were sampled with 5 cm dia Dutch augers. Soil characteristics are given in Table 1.

Average daily soil temperature at sampling time (17 September 1996) was 15.5°C in the top 10 cm layer. The soils were then hand-picked at this temperature (±1°C) to remove any obvious plant or animal residues, then sieved at field moisture content (< 2 mm). A portion of both soils was sampled and analysed for their chemical properties just after sieving, while the remaining soils were adjusted to 40% Water Holding Capacity (WHC) prior to incubation.

2.2. Incubation conditions

Immediately after adjustment of soil moisture (Section 2.1) the moist soil samples were incubated at six different temperatures (0, 5, 10, 15, 20 or 25°C) in the dark, under aerobic conditions at 40% WHC. For each temperature three separate plastic bags, each containing about 1.5 kg moist soil were prepared. A single replicate was then removed from each bag for biomass and ATP analysis as required. Biomass C and soil ATP contents were determined at 1, 3, 6, 13 and 23 days of incubation except that soils incubated at 10 and 20°C were analysed only once — after 23 days of incubation.

2.3. Analytical measurements

Biomass carbon (Bc) was measured by the Fumigation–Extraction method (Vance et al., 1987). Moist soil portions, equivalent to 25 g oven dry soil, were fumigated with ethanol-free chloroform for 24 h. The soils were then extracted with 100 ml 0.5 M $K_2SO_4$for 30 min. A set of non-fumigated soils extracted similarly at the time fumigation commenced. Organic C in the soil extracts was measured by an automated UV-persulphate oxidation procedure (Wu et al., 1990).

Soil ATP was extracted from soil by the TCA reagent, a mixture of 0.5 M trichloro-acetic acid, 0.25 M phosphate and 0.1 M paraquat (Jenkinson and Oades, 1979). In order to estimate the amount of ATP lost in the soil during extraction, for example by adsorption onto soil colloids and by hydrolysis, two different soil extractants were used. Extractant A contained the reagent only, while Extractant B was prepared as Extractant A except that 10 ml of 1 mM ATP l^{-1} was added per litre of Extractant A as the hydrated tetrasodium salt (SIGMA) as internal standard. This gave a final ATP concentration (as the free acid) in Extractant B of 50 pmol 50 μl^{-1}.

The ATP concentrations in the soil extracts were determined by the fire-fly luciferin-luciferase assay system (Tate and Jenkinson, 1982a). Luciferin was supplied by SIGMA while purified luciferase was supplied by Boehringer-Mannheim. The luciferase was dissolved in 1 ml 0.5 M Tris–acetate buffer at pH 7.5, then this concentrated solution was stored frozen at −18°C in 50 μl aliquots in glass vials. (The enzyme remains stable for several months under these conditions). Just before use an aliquot of concentrated enzyme solution was thawed and diluted with 60 mM Tris–albumen buffer (10 ml, pH 7.95). The luciferin was dissolved in water to give 500 μg ml^{-1}, then added to the enzyme solution and stored over ice.

Counting commenced about 10 min after enzyme addition. However, with arsenate buffer, the exact time of the start of counting after enzyme addition is relatively unimportant. Indeed, we have obtained perfectly valid, but smaller, counts more than 12 h after enzyme addition. Bioluminescence was measured using a Packard liquid scintillation counter set out of coincidence. Counts over a 6 s integration time were compared with a standard curve of ATP prepared in Extractant A.

All results are expressed on an oven-dry soil basis (105°C, 24 h) and are means of three replicate measurements.

2.4. Statistical analyses

Standard errors were calculated using approximate error propagation formulae and are given as standard errors of the differences of the means. Regression analysis and analyses of parallelism were carried out using Genstat.

3. Results and discussion

3.1. Changes in biomass C during incubation at different temperatures

Biomass C in the arable Soil 1 remained constant at around 275 μg biomass C g^{-1} soil within the temperature range of 0 to 25°C until the end of the incubation on day 23 (Fig. 1). In contrast, biomass C apparently increased rapidly in the grassland soil, from about 910 μg biomass C g^{-1} soil just after sampling to 1175 μg C g^{-1} soil by the end of day 1. After this, it remained constant up to day 23, when the experiment ended.

We have great doubt as to the validity of this apparent large and rapid increase in biomass in such a short time and under conditions where no extraneous substrates were applied. While we have observed similar, unaccountable, initial increases under comparable conditions (e.g., Joergensen et al., 1990), there could simply not be a large amount of readily-assimilatable substrate present to support such huge biomass increases. Chander and Brookes (1991) reported biomass synthesis efficiencies of about 11% following addition of 1000 μg maize–C g^{-1} soil 5 days after addition. Thus this large substrate input only produced an additional biomass of about 110 μg C g^{-1} soil, far less than that reported here. The most likely explanation is an artefact in the methodology (probably because the grassland soil was rather dry) just after incubation commences and which fortunately declines in effect very quickly (Sparling and West, 1989). The effect was not detected in the arable soil, probably because of its higher moisture content when sampled.

After the initial apparent increase, the biomass size remained constant, with no significant differences

Table 1
Soil characteristics

Soil no.	Management	Sampling depth (cm)	pH (H_2O)	Clay content (%)	Organic C (mg g^{-1})	Total N (mg g^{-1})
1	Arable	0–23	6.1	26	16.5	1.72
2	Grassland	0–10	6.0	26	34.5	3.10

between temperatures. The apparent decreases in biomass by day 23 at 15 and 25°C were not statistically significant. Thus the biomass in both soils was insensitive to temperature over the comparatively large range from 0 to 25°C. This also suggests that the decomposability of soil organic matter was little, if at all, increased with increasing temperature or presumably there would have been some net synthesis of biomass. Since biomass measurements are often done after a soil has received a conditioning incubation, usually of several days at 25°C, it is reassuring to observe that there were no changes in biomass concentration during this period in either soil, irrespective of the incubation temperature. Of course, an alternative explanation is that the decline in biomass at the higher temperatures was balanced by an increase in the synthesis of new biomass due to enhanced decomposability of the soil organic matter. To investigate that possibility would fall outside the scope of our study.

3.2. Changes in soil ATP concentrations during incubation

Soil ATP concentrations did not change markedly during the incubations. The ATP content of the arable soil measured just after sampling and sieving was about 2.2 nmol ATP g^{-1} soil. While there were some fluctuations, the only really consistent change was a slightly larger ATP concentration on days 13 and 23 at 25°C (Fig. 2a). This gave a final ATP concentration of 3.11 nmol ATP g^{-1} soil at 25°C, an increase of about 40% compared to the initial ATP concentration.

The grassland soil apparently behaved somewhat differently, although this was probably in part because initial soil ATP concentrations were larger than in the arable soil and so changes were easier to detect (Fig. 2(b)). From an initial value of 6.2 nmol ATP g^{-1} soil, it increased to 8.4, 9.4, 10.4 and 11.2 nmol ATP g^{-1} soil, respectively, at 0, 5, 15 and 25°C after 23 days of incubation, giving a maximum percentage increase of 81% at 25°C. A similar ATP response to temperature occurred in forest humus. Arnebrant and Bååth (1991) reported that incubation at 25°C for 1–7 days gave ca. 50% larger ATP values than at 4°C.

While the biomasses in both of our soils showed an increase in ATP with increasing temperature, the increases were relatively small, not even doubling between 0°C and 25°C in either case. This, together with the biomass data, indicates that, at any given temperature, ATP is largely a measure of soil biomass content. However, ATP tends much more towards an

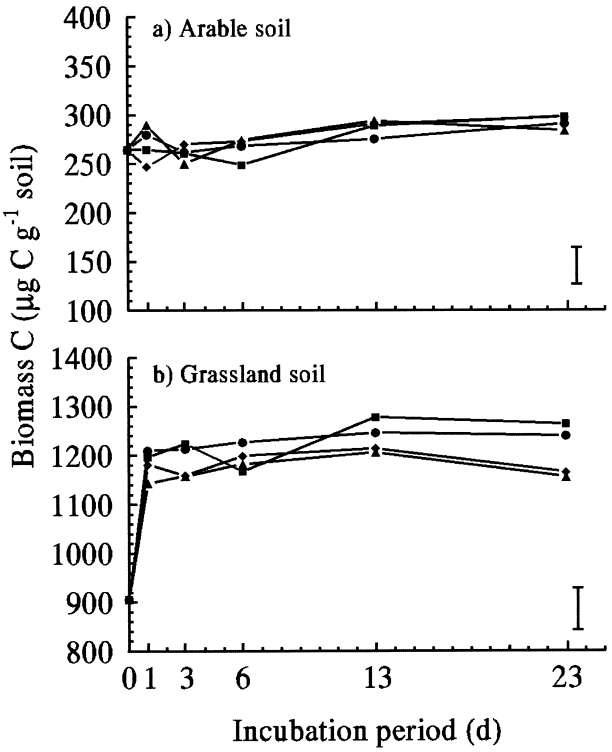

Fig. 1. Changes in biomass C concentration in soils incubated at 0 (■), 5 (●), 15 (◆) and 25 (▲) °C for up to 23 days. Bars show mean 95% confidence limits (39 and 90 µg biomass C g^{-1} soil, respectively) for (a) arable and (b) grassland soils (n = 3).

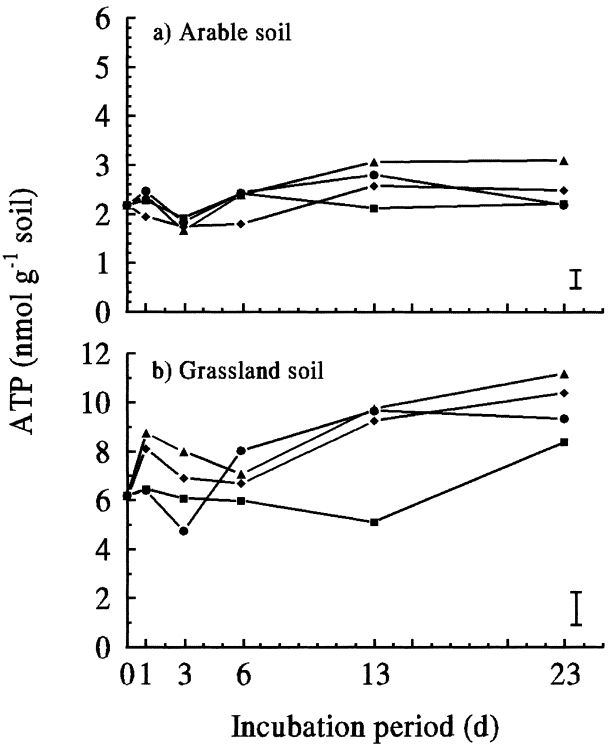

Fig. 2. Changes in ATP concentrations in soils incubated at 0, 5, 15 and 25°C for up to 23 days. Bars show mean 95% confidence limits (0.4 and 1.4 nmol ATP g^{-1} soil, respectively) for (a) arable and (b) grassland soils (n = 3). (Symbols: same as Fig. 1.)

activity measurement with changing soil temperature, unlike soil biomass C.

It is noteworthy that there were generally increases in ATP following incubation at higher temperatures while biomass C did not change significantly. Tate and Jenkinson (1982b) similarly reported that ATP increased by 73% but biomass C by only 23% when a UK grassland soil was incubated for 7 days at 25°C. The comparable increases at 10°C were 16% for biomass C and 19% for ATP. These relatively small increases in total (rather than percentage) ATP do not therefore reflect much change in biomass C (Fig. 1) but rather increases in ATP synthesis or adenylate energy charge (AEC), or both.

The increases in soil ATP concentration with increasing temperature in both our soils means that if ATP is to be used as an index of biomass then, for maximum accuracy, the ATP must be measured at a standard temperature. It is much less critical that biomass C measurements are done by FE at a standard temperature, apparently even within the comparatively wide range of 0–25°C. There is no experimental reason why 25°C should not be the chosen incubation temperature for measurement of biomass C (Jenkinson and Powlson, 1976) and ATP as proposed by Jenkinson and Oades (1979).

In complete contrast to our findings is the work of Eiland (1985). He reported 4- to 5-fold shifts in soil ATP concentrations at three soil depths between 0 and 20 cm in a fallow garden soil in Denmark over 24 h. Soil temperature in the 0–3 cm depth ranged from about 2 to 12°C and from 3 to 7°C at the 10–20 cm depth. Overall, the changes in soil ATP concentrations closely followed the fluctuations in soil temperature. We cannot reconcile the differences between our results and those of Eiland (1985). Certainly, the daily temperature fluctuations reported by Eiland (1985) were very much greater than others reported for Northern European soils (e.g., Hay, 1977).

3.3. Changes in biomass ATP concentrations with temperature

Knowles (1977) listed the ATP concentrations and AECs of more than 50 micro-organisms growing in culture. Selecting data only from those with an AEC above about 0.75 (i.e., organisms which would be considered to be at a high rate of metabolic activity), 21 cultures of prokaryotic micro-organisms had a mean ATP concentration of 8.0 ± 0.42 (standard error) μmol ATP g^{-1} C, assuming dry cells contain 45%C. Similar biomass ATP concentrations were reported for eukaryotic microbes in vitro by Knowles. Karl (1980) reported that both exponentially growing prokaryotes and eukaryotic micro-organisms had mean ATP concentrations within the range of about 4.5 to 13 μmol ATP g^{-1} biomass C (median value around 8 μmol ATP g^{-1} biomass C). These microbial biomass ATP concentrations of micro-organisms grown in vitro therefore compare closely with those measured initially in the microbial biomasses of the arable (8.2 μmol) and grassland soils (6.8 μmol ATP g^{-1} biomass C). The measurements were made within 1.5 h of soil sampling. The field soil temperature was 15.5°C at the time of sampling and the soils were kept at this temperature ±1°C at all soil preparation stages prior to setting up the main incubation experiment (Section 2.2).

During the incubation the soil microbial biomass ATP concentrations ranged from about 5 to 12 μmol ATP g^{-1} biomass C, but trends between biomass ATP concentrations and incubation times did not become readily apparent up to about day 13 (results not given). However, by the end of day 23 of incubation, biomass ATP concentrations were positively and linearly related to temperature: Biomass ATP concentration (μmol ATP g^{-1} biomass C) = $6.98 \pm 0.35 + 0.134 \pm 0.023\ T^0$ ($r^2 = 0.77$). There was no significant difference in the slope between the regressions of the arable and grassland soil. The difference in the intercept between the regression of the arable and grassland soil was statistically significant but was not large enough to be meaningful scientifically. It was small (0.85) compared to the errors of the values we are measuring. For simplicity, we used a single line to represent the relationship between temperature and microbial biomass ATP concentration (Fig. 3). At 25°C the biomass ATP concentration, meaned between the arable and grassland soils, was 10.3 μmol ATP g^{-1} soil. This is very similar to the mean value of 11.7 μmol ATP g^{-1} biomass C reported for the soil microbial biomass by Jenkinson (1988) who collated all

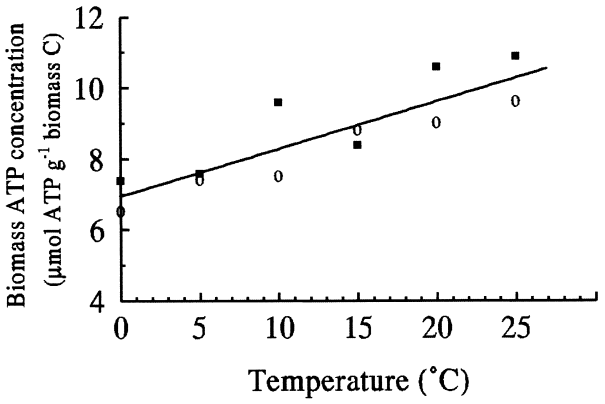

Fig. 3. Biomass ATP concentrations in the arable (■) and grassland (○) soils incubated at 0, 5, 10, 15, 20 and 25°C after 23 days of incubation. Biomass ATP concentration (μmol ATP g^{-1} biomass C) = $(6.98 \pm 0.35) + (0.134 \pm 0.023)\ T^0$.

appropriate data then published in the world literature to obtain this value.

It seems quite remarkable that the soil microbial biomass ATP concentrations should be so comparable, especially when comparing analyses taken from the world-wide literature (Jenkinson, 1988) with our two UK soils. It seems even more remarkable that the ATP concentrations of both prokaryotic and eukaryotic micro-organisms growing exponentially in vitro should also compare with the, mainly, dormant (Jenkinson and Ladd, 1981) soil microbial biomass. Indeed, even the mean value of about 7 μmol ATP g^{-1} biomass C at 0°C (Fig. 3) fits comfortably within the range of ATP concentrations reported by Knowles (1977) and Karl (1980) for micro-organisms growing exponentially in vitro.

The soil microbial biomass certainly has the capacity to alter its ATP concentration under other circumstances. For example, Brookes et al. (1983) reported that ATP declined from about 77–28% of total adenine nucleotide content (A_T) when field moist soil was air-dried, but increased to 67% of A_T within 2.5 h of rewetting to the original soil moisture content. Similarly, the ATP contents of a set of previously aerobic soils declined to between 32% and 64% of the original ATP contents during 20 days of anaerobic incubation under water. However, on aeration for 1 h, ATP increased to between about 70% and 84% of the values in the original aerobic soils (Inubushi et al., 1989).

Why the biomass does not adopt a similar strategy in aerobic soils at low temperatures is unknown. It seems certain that the turnover rates of ATP will increase with increasing temperature (Karl, 1980) even if changes in total amounts of ATP are comparatively small, certainly much smaller than the increases predicted from the Q_{10} rule.

Joergensen et al. (1990) also reported that ATP concentrations were similar to each other in a grassland soil incubated at 15 or 25°C for up to 240 days, with mean declines of 0.11% and 0.22% per day. In contrast, the ATP concentrations in other samples of the same soil incubated at 35°C declined precipitously, at 1.72% per day for the first 50 days. Taking all results (except those at 35°C) over the 240 days incubation, the mean biomass ATP concentration was 11.9 μmol ATP g^{-1} biomass C, close to that previously observed under laboratory conditions when moist soils (40% WHC) were analysed immediately following a conditioning incubation of several days at 25°C (e.g., Oades and Jenkinson, 1979; Jenkinson, 1988).

Despite a wide-ranging literature review, we found no comparable studies to ours, even in chemostat experiments, other than those which we report here. The general picture that emerges from our work and that of most others is that the total biomass concentration in soil is little, if at all, altered by temperatures between 0°C and 25°C. Soil ATP concentration, however, does gradually increase with increasing temperature. The increase is not due to increased synthesis of biomass C but rather due to either an increase in AEC or de novo synthesis of ATP (or both). Thus, in our work the biomass ATP concentration increased from about 7 to 10 μmol ATP g^{-1} biomass C within the above temperature range, when measured on day 23 of incubation. It did not therefore deviate from the range of microbial ATP concentrations considered typical of micro-organisms which are growing exponentially (e.g., Karl, 1980; Knowles, 1977) at either end of our temperature range. However, although ATP concentrations are held almost constant it is possible that some microbial groups become active and others become inactive as the temperature changes.

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